PHOTOHYDRATION OF TESTOSTERONE AND 4-ANDROSTENE-3,17-DIONE
IN AQUEOUS SOLUTION (1)

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ABSTRACT

Irradiation of testosterone, 4-androstene-3,17-dione, or their "half-molecule" 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3H)-naphthalenone in dilute aqueous solutions with ultraviolet light of 254 nm wavelength caused rapid addition of water across the olefinic bond with formation of 5,17β-dihydroxy-5α-androstan-3-one, 5-hydroxy-5α-androstane-3-17-dione, and 9-hydroxy-10-methyl-2-decalone, respectively. Time-lapse spectrometry in the ultraviolet region showed that the photohydration of the androgenic steroids was extremely efficient and virtually free of side reactions. Preparative-scale photolytic reactions carried out in water-methanol solutions allowed isolation and characterization of photoproducts.

When irradiated with ultraviolet light, many steroids undergo a variety of photochemical transformations such as dimerization, addition, or rearrangement (4). Most of these photoreactions have been studied in organic solvents primarily because steroid compounds tend to have limited solubility in water. However, both natural and synthetic steroids express their physiologic or pharmacologic properties in aqueous medium at very low concentration. Therefore, we have explored the photochemical reactions of some steroids in dilute water solutions. Since the human skin has distinct transparency to ultraviolet light (5), such reactions may indeed take place in vivo on exposure to sun or artificial ultraviolet sources.

In this paper we describe the photohydration of testosterone I and 4-androstene-3,17-dione II to 5,17β-dihydroxy-5α-androstan-3-one III and 5-hydroxy-5α-androstane-3,17-dione IV, respectively, when irradiated with light of 254 nm in dilute aqueous solution.

The reactions were found to be extremely efficient and photochemically selective, since the respective products exhibit only minor light-absorption at the exciting wavelength when compared to the reactants.
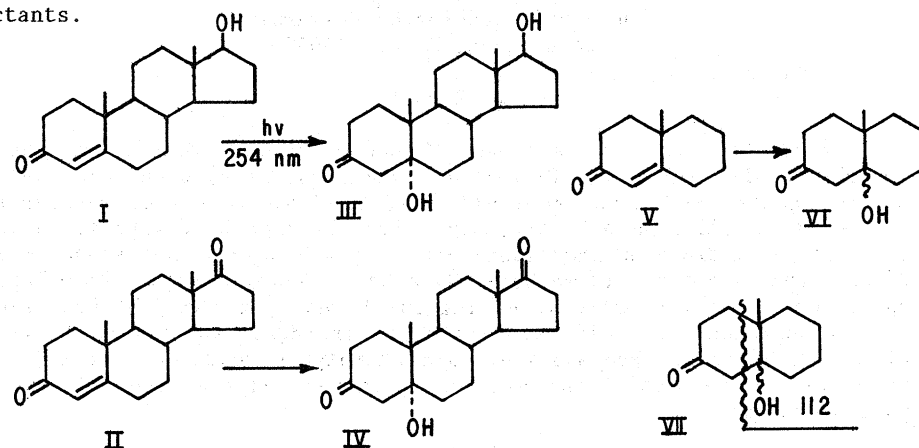
The bicyclic enone 4,4a,5,6,7,8-hexahydro-4a-methyl-2(3H)-naphthalenone V was included in this study because it represents the common "half" of the two main androgenic hormones I and II. Since, as we will show below, the photohydration and photoreduction affect only the A and B rings of these steroids, enone V is an appropriate choice of "model" molecule offering a simplified analytical task in characterizing photoproducts.

RESULTS

Time-lapse Spectrometry (TLS) (6). As one would expect, all three α,β-unsaturated ketones have similar ultraviolet absorption spectra in the 200-400 nm range. They exhibit a relatively intense absorption band centered around 245 nm with extinction coefficients of 1.5-1.7 x 10^4 1 mole^{-1} cm^{-1} and a much weaker shoulder in the 280 to 310 nm region. Undoubtedly the nearly-gaussian band at 245 nm is associated

with the lowest electronic transition in the conjugated enone chromophore of ring A while the longer-wavelength absorption above 280 nm (ε<100 mole^{-1}cm^{-1}) represents n$\rightarrow\pi^*$ promotion in the carbonyl group.

When dilute ($\sim$5 x 10^{-5} M) water solutions of enones $\underline{I}$, $\underline{II}$, or $\underline{V}$ were submitted to successive short-interval irradiation at 254 nm with a 9W low-pressure Hg handlamp, the $\pi\rightarrow\pi^*$ absorption band diminished progressively while the n$\rightarrow\pi^*$ region remained unchanged, as shown for $\underline{I}$ in Figure 1. It is quite clear that the α,β-unsaturated carbonyl chromophore in the reactant was converted to a group exhibiting no significant absorption in the near-ultraviolet spectrum in the photoproduct. While the total irradiation time required for completion of the reaction of enone $\underline{V}$ was 8 minutes, the two steroids needed only 90 seconds for total conversion under the same experimental conditions and concentration. The time-lapse spectrometry experiments at low concentrations showed also that this extremely efficient reaction was not complicated by significant side or consecutive reactions.

Product Characterization. Since the title compounds have only a limited solubility in water, preparatory-scale photolyses were carried out in 80:20 water:methanol (v:v) solutions. Even in this solvent mixture, the maximum concentrations attainable were about 3 x 10^{-3} M. The solutions were irradiated with 254 nm light and the extent of conversion was estimated from uv spectra of diluted aliquots. The gasliquid chromatogram of methylene-chloride-extracted material showed unreacted enone, a main photoproduct, and several side-products. Analytical samples of the main products were obtained using preparatory-scale glc columns.

The main photoproduct from enone $\underline{V}$ showed only weak absorption in the uv at 280 nm. Its infrared spectrum showed a characteristic OH peak absorption at 3610 cm^{-1} and an unconjugated carbonyl group (v_{max} = 1710 cm^{-1} with shoulder at 1732 cm^{-1} (7)). The resonance signals in the nmr spectrum were at fields higher than 3 ppm, with no absorption in the vinyl-hydrogen range. The mass spectrum showed the molecular ion at 182 m/e (10%), a 25% peak at M^{+}-18 = 164, an ion of mass corresponding to $\underline{V}$, and a base peak at 112 m/e. These analytical data are consistent with 9-hydroxy-10-methyl-2-decalone structure $\underline{VI}$.

The addition of water to the C=C of enone $\underline{V}$, indicated by the molecular weight of the product and confirmed by its peak at M^{+}-18 m/e in the mass spectrum, could take place in two ways. That the hydroxyl group was in fact attached to the C_9 atom was clearly shown by the peak of the most abundant ion at m/e 112 in the mass spectrum. Only the 9-hydroxyketone, but not its 1-hydroxy isomer, can give rise to a 112 m/e stable fragment, as shown in $\underline{VII}$. The actual ion formed from this fragment is 2-methylcyclohexanone. This was established in a separate experiment in which 2-methylcyclohexanone was found to give a stable molecular ion upon electron impact. Attachment of the hydroxyl group to the C_9 atom is also suggested by the ir absorption band at 3610^{-1} which is characteristic of a tertiary OH (7).

The uv, ms, and nmr spectra of the main photoproducts $\underline{III}$ and $\underline{IV}$ from testosterone and 4-androstene-3,17-dione, respectively, were essentially analogous to those shown for hydroxydecalone $\underline{VI}$ above. Their molecular weights were also 18 amu higher than the corresponding precursors, and they showed prominent peaks at M^{+}-18 m/e in the mass

spectrum, consistent with initial photohydration and fragmentation in the mass spectrograph with ready loss of a neutral water molecule. The nmr spectra of both III and IV exhibited a broad band between 0.6 ppm and 2.9 ppm (δ vs TMS). Singlet methyl resonances were observed at 0.78 ppm and 1.16 ppm for III and 0.90 ppm and 1.18 ppm for IV. A broad doublet due to the methine hydrogen in III was observed at 3.6 ppm. The A ring chromophores of both III and IV exhibited positive Cotton effects, with bands centered at 290-300 nm in their circular dichroism (CD) spectra, suggesting the α configuration for the hydroxyl group at the 5-carbon (8).

Significant amounts of a photoproduct other than the water adduct were formed in the prep-scale reactions of both I and II in methanol-water solution. The quantity of secondary product was almost equal to that of the main hydroxyketone photoproduct. After appropriate chromatographic isolation, these side products were authenticated by comparison of their mass spectra with those of commercially available samples. Thus, the side product from I was identified as 17β-hydroxy-5α-androstan-3-one and that from II as 5α-androstane-3,17-dione.

Photolysis of Isomeric Methylhexahydronaphthalenones. Two bicyclic enones, VIII and X, were thought to present interesting configurational variations with respect to I, III, and V. They differ from the isomeric enone V only by the position of the methyl group. The behavior of these two isomers of V on uv irradiation in dilute aqueous solutions was also explored.

$$\underset{\text{VIII}}{} \longrightarrow \underset{\text{IX}}{} \quad \underset{\text{X}}{}$$
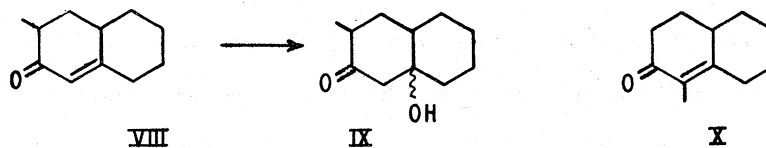
Both the TLS diagram and the preparative scale results with derivative VIII were comparable to those presented for V. There is little doubt that photohydration with formation of IX took place. However, the analgous phototransformation of enone X on photolysis in water was much less efficient than that of V. It required exposures several times longer for conversions and tended to form larger amounts of side-products as shown by both the TLS diagram and the glc analysis.

DISCUSSION

α,β-Unsaturated ketones undergo a variety of photochemical reactions including α-cleavage, reduction, dimerization, cycloaddition, rearrangements, and reaction with solvents (4). Although light-induced additions of many molecules to the olefinic bond of conjugated cyclo-alkenones have been reported (9), including solvents (10, 11) and groups from adjacent substituents (12), the efficient photoaddition of water, apparently, has not been reported.

The photohydration reaction reported here for I and II in dilute aqueous solution is extremely efficient, with complete conversion in less than one minute total exposure to a weak 9 W shortwave uv source.

Formation of side-products other than the hydroxyketones in preparative-scale irradiations was not too surprising since the solvent

was aqueous methanol, not water. The formation of 5α-dihydrotestos-
terone and 5α-androstane-3,17-dione by photoreduction of the alkene
group is known to take place in alcoholic solvents (13).

The stereochemistry of the bicyclic enone and the site of attach-
ment of the methyl substituent have a marked effect on the efficiency
of photohydration. Incorporating the bicyclic enone configuration in
the extended polycyclic structures of testosterone or 4-androstene-
3,17-dione seems to cause a many-fold increase in the efficiency of
water photo-addition. It is not inconceivable that the added rigidity
in the tetracyclic steroid structure diminishes the rate of thermal
deactivation in the excited enone. It seems that the deactivation
competing with the photoreaction requires twisting of the enone site
(14).

Repositioning the angular methyl substituent in V to the non-
olefinic α-position in VII did not seem to affect significantly the
outcome or the efficiency of the photohydration reaction. On the other
hand, the attachment of a CH_3 group in the vinylic α-position in X
caused not only marked decrease in efficiency but also formation of
much larger amounts of side-products. Such strong influence of direct
substitution on the olefinic moiety of the enone has also been noted in
other photoreactions (15).

The photochemical addition of water to enones I, II, and V yielded
hydroxyketones with the OH group attached to the β-carbon. This con-
trasts with the photoaddition of methanol to α,β-cycloalkenones in
which the methoxy group was reported to occur at the α-carbon (16).
The different site of attachment of the hydroxy vs. the methoxy group
in the respective photoproducts suggests different mechanistic
pathways.

$$\text{XI} \quad \xrightarrow{H_2O} \quad \text{XII} \quad \longrightarrow \quad \text{XIII} \quad \longrightarrow \quad \text{XIV}$$
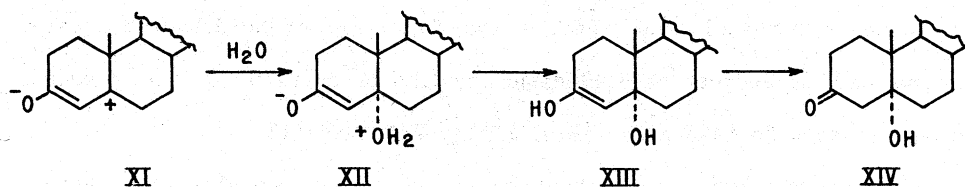
A simple and reasonable mechanism for the photohydration is shown above. The excited state of the enone responsible for the addition reaction is represented as dipolar structure XI in a customary way (2). Addition of water at the electrophilic β-carbon atom generates zwitterion XII which readily undergoes proton exchange to enol XIII and then tautomerization to β-hydroxyketone XIV.

EXPERIMENTAL

Bicyclic enone V was synthesized from 2-methylcyclohexanone and 4-diethylamino-2-butanone methiodide by a known procedure (15). Testosterone and 4-androstene-3,17-dione were obtained from Research Plus Laboratories. Bicyclic unsaturated ketones VIII and X were purchased from Aldrich Organic Chemicals. Uv-visible spectra were recorded on a Cary Model 14 spectrometer and ir spectra on a Perkin-Elmer 467 spectrometer. Mass spectra were determined with a LKB Model 9000 spectrometer which was attached to the gas-chromatograph. Glc separations were carried out on a polar 6 feet x 1/8 inch column with 3% Triton X-305 on Chromosorb W for 10-methyloctalone and on a 4 feet x 1/8 inch column packed with 3% JXR on Gas Chrom Q for the steroids, with helium carrier. Nmr spectra were recorded on a JEOL-60QFX Fourier-transform spectrometer at 60 MHz. CD spectra were determined on a JASCO-J41C spectropolarimeter. The TLS experiments were carried out in 1 cm quartz cells irradiated with a 9 W Ultraviolet Products handlamp. Preparative-scale photolyses were done in a home-built reactor.

Preparative-scale irradiation of bicyclic enones V, VIII, and X were carried out with 40 ml of 6 x 10^{-3} M water:methanol (80:20) solutions in the immersion-well apparatus to approximately 50% reactant conversion for 1.5, 1.5, and 12 hours, respectively. The resulting reaction mixtures were extracted with methylene chloride, dried, and separated by glc in the 140 to 180°C range.

Forty ml of 10^{-3} M solutions of testosterone or androstenedione in water:methanol (80:20) mixture were irradiated with a low pressure Hg arc lamp in the reactor for a total of 20-30 minutes. The extent of conversion was followed by recording the uv spectra of diluted aliquots. The photolyzed solutions were extracted with methylene chloride, dried over anhydrous $MgSO_4$ and analyzed by passing this solution through the glc - mass spectrometer.

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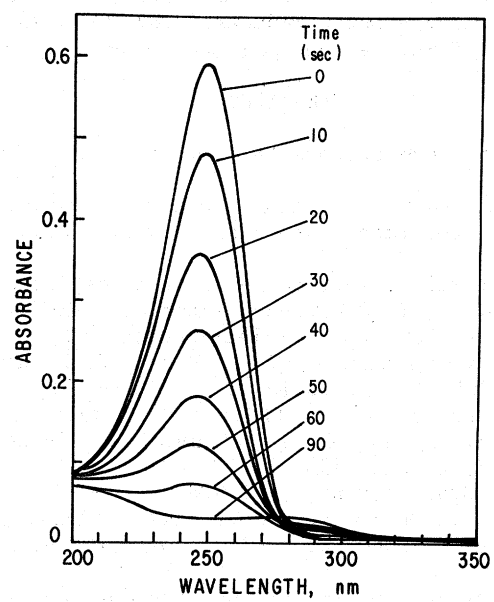16. R. Noyori and M. Kato, Bull. Chem. Soc. Japan, 47, 1460 (1974).

Figure 1. Photolysis of testosterone
4×10^{-5} M in water at 254 nm.